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Prevalence of extended-spectrum beta-lactamase producing gram negative isolates in Enterobacteriaceae group of bacteria in clinical samples from a tertiary care hospital

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Abstract

Introduction: Extended spectrum β-lactamases (ESBLs) are enzymes that intervene resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and but do not affect carbapenems (e.g., meropenem or imipenem). Though the number of ESBLs producing organism has been increasing day by day, the detection methods and treatment option for them are extremely limited.

Objective: Objective of the study was to investigate the rate of ESBLs production and their antibiotic susceptibility pattern.

Materials and method: A total 200 Gram negative isolates from various clinical samples received in microbiology laboratory, Sir Takhtsinhji General Hospital, Bhavnagar were studied and Antibiotic susceptibility test was done for commonly used antibiotics. A hospital-based study was conducted in microbiology laboratory, Sir Takhtsinhji General Hospital, Bhavnagar from February 2012 to August 2012. A total of 200 Gram negative isolates from various clinical samples were collected and identified using the conventional biochemical tests following the Clinical and Laboratory Standard Institute (CLSI) guidelines. Antimicrobial susceptibility testing (AST) was performed using the standardized Kirby-Bauer disk diffusion method.

Results: Among the total isolates 89(44.5%) were ESBLs producer, and the rate of ESBLs positivity was 39.8% for *E. coli* (33 out of 83), 10% for *Proteus mirabilis* (1 out of 10), 51.4% for *Klebsiella* spp (55 out of 107). ESBLs producing organisms were resistant to most of the antibiotics but 100% were sensitive to imipenem, meropenem, and cefoperazone + sulbactam.

Conclusion: Screening for ESBLs production requires to be carried out regularly in all clinical diagnostic laboratories to direct clinicians in appropriate selection of antibiotics.

Keywords: β-lactamases; ESBLs; multidrug resistance; Kirby-Bauer disk diffusion; *E. coli*, *K. pneumonia; Proteus mirabilis*

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Introduction

Beta-lactam antimicrobial agents are the most commonly used treatment of bacterial infections [1]. Resistance to beta-lactam antibiotics among clinical isolates of gram-negative bacilli is most often due to the production of beta-lactamases [1, 2]. These enzymes are numerous and they mutate continuously in response to heavy pressure of antibiotic use and have tending to the development of extended spectrum beta-lactamases (ESBLs) [3]. Many of these ESBLs have evolved from the TEM-1, TEM-2, and SHV-1 beta-lactamases that are widely distributed among the Enterobacteriaceae [4-6].

ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporin (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., imipenam or meropenam) [7, 8]. These ESBLs are commonly inhibited by beta-lactamase-inhibitors such as clavulinic acid, sulbactam and tazobactam [5, 6].

ESBLs were first identified in 1983 [9]. National Committee for Clinical Laboratory Standards (NCCLS) guidelines recommend screening all *K. pneumoniae, K. oxytoca*, and *E. coli* for which minimum inhibitory concentrations (MICs) of cefpodoxime, ceftazidime, cefotaxime, ceftriaxone, and aztreonam are ≥2 μg/m [8]. The organism can be produce ESBL against one or more of the above listed antibiotics. Various conventional or automated laboratory methods are available to detect this. Among Gram-negative bacteria, the emergence of resistance to extended-spectrum cephalosporin has been a major concern. Treatment of infections caused by ESBL-producers is complicated not only by resistance to extended-spectrum cephalosporin, but also because many ESBL genes are on large plasmids containing genes which also encode resistance to many other antibiotics including aminoglycosides, chloramphenicol, sulfonamides and tetracycline antibiotics [8, 10] . These infections have a significant impact on patient's mortality and additional financial burden [11]. The most common method of testing for ESBLs is screening for reduced susceptibility to cefpodoxime/ cefotaxime/ ceftriaxone/ ceftazidime followed by phenotypic confirmatory testing by demonstrating a synergistic effect between an indicator cephalosporin and a beta lactamase inhibitor i.e., clavulanic acid [12, 13].

Due to much significance in clinical conditions and high resistance pattern of these ESBL producer pathogens, it is important to detect strains producing ESBL, so this study was undertaken to detect the occurrence of ESBL producing pathogens among various clinical isolates of Enterobacteriaceae in sir Takhtsinhji Hospital, Bhavnagar, tertiary care hospital of Gujarat.

The Objectives of this study were to detect extended spectrum beta lactamases in Gram negative bacilli of Enterobacteriaceae group isolated from various clinical samples received in microbiology laboratory, Sir Takhtsinhji General Hospital, Bhavnagar, to know the percentage of extended spectrum beta lactamases producing strains of gram negative bacilli of Enterobacteriaceae group isolated from different clinical samples and to know the treatment alternative for the ESBL producer clinical isolates.

Materials and methods

Study design

A hospital-based study was conducted from February 2012 to August 2012 at Microbiology Department, Government Medical College and Sir Takhtsinhji General Hospital, Bhavnagar, India. Different clinical samples like urine, pus, blood culture, sputum and body fluids (ascetic fluid, pleural fluid etc.) taken from patients admitted in the Sir Takhtsinhji General Hospital, Bhavnagar. Proper information about sample collection was given in the case of urine and sputum. Samples which are improperly collected or those having lack of proper labeling were excluded from the study. We had included all age groups and both genders.

Isolation and identification of the isolates

A total of 200 clinical samples [urine, 101(50.5%); sputum, 6(3%); pus, 74(37%); blood culture, 16(8%); and body fluids, 3(1.5%)] were processed (cultured) following the Clinical and Laboratory Standards Institute (CLSI).

These samples were processed as per CLSI guidelines on MacConkey agar as well as on blood agar. These plates were incubated for 24 hours at 37°C. After 24 hours, the plates were checked for colony

morphology. These were followed by Gram stain and motility and identification of bacteria and sensitivity by modified Kirby-Bauer method on Mueller Hinton agar were done as per CLSI Guidelines [12, 13]. After looking for colony morphology & characteristics Gram stain and motility of organisms were carried out as shown in Table 1. Then these isolates were subjected for biochemical reaction as shown in Table 2.

Table 1: Gram stain and motility of organisms.

Organisms	*Gram stain*	*Motility*
E. coli	Gram negative short rod	Sluggishly motile
K. pneumonia	Gram negative short, plump, straight rod. Capsule often prominent and can be seen in as haloes around rod.	Non-motile
Proteus mirabilis	Gram negative coccobacilli	Actively motile

Table 2: Biochemical reactions test.

Biochemical reactions test	*E. coli*	*K. pneumonia*	*Proteus mirabilis*
Catalase test	Positive	Positive	Positive
Indole test	Positive	Negative	Negative
Methyl red	Positive	Negative	positive
Vogues Prokaur's test	Negative	Positive	Negative
Citrate test	Negative	Positive	Negative
Urease test	Negative	Positive	Positive
Triple sugar iron test	A/A/-/-	A/A with gas	Alk/A with H2s

Antibiotic susceptibility test

After confirming the isolate by biochemical reaction, Antibiotic Susceptibility Testing (AST) was performed by the Kirby-Bauer disk diffusion technique using Muller Hinton agar (MHA) according to the CLSI recommendations [12, 13]. The antibiotic disks tested were ceftazidime (CAZ), cefuroxime (CFR), cefoperazone (CPZ), cefotaxime (CTX), ceftriaxone (CTR), amikacin (AK), gentamycin (GEN), ampicillin/sulbactam (AS), tetracycline (TE), ciprofloxacin (CIP), imipenam (IPM), meropenam (MRP). The zones of inhibition were interpreted and measured as sensitive or resistant according to Clinical and Laboratory Standards Institute guidelines [12, 13].

Screening and confirmation of ESBL producers

For screening the ESBL producing isolates, pure culture suspension of isolates in peptone water was prepared and incubated the tubes at 37°C for 2-4 hours. Then compared it to the 0.5 McFarland standard suspensions followed by inoculating this suspension on Muller Hinton agar with sterile cotton swab by lawn culture method. Then placed the antibiotic disks of third generation cephalosporin and incubate the plate at 37°C for 18-24 hours and measured the zone of inhibition for all drugs and compare that with CLSI guidelines. We have selected those samples which were resistant to third generation cephalosporin [12].

Inoculate the Muller Hinton agar with standardized inoculum (0.5 McFarland's standard) to form a lawn culture by disk diffusion method. With sterile forceps, the disks of cefoperazone (75µg) and cefoperazone plus Sulbactam (75µg + 10µg) were placed at the recommended distance (20mm) from each other on the plate. Then incubated the plate at 35±2°C in ambient air for 16-18 hours.

Interpretation

Organism was considered as ESBL producer if there was a 5mm or more than 5mm increase in the zone diameter of cefoperazone plus sulbactam compare to the cefoperazone alone. The isolates showing positive and negative phenotypic double diffusion test respectively.

Statistical analysis

Data were analyzed using MS Excel. Descriptive data were expressed as a percentage. Chi square test was conducted with a P value of ≤0.05 for the association bacteria and source of samples to be considered significant.

Results

A total of two hundred isolated Gram-negative bacteria. Different samples (urine, pus, blood, sputum and body fluids, ascetic fluid, pleural fluid etc.) were collected out in the Microbiology Department, Government Medical College and Sir Takhtsinhji General Hospital, Bhavnagar and subjected for the ESBL detection. Table 3 shows that out of 200 Gram-negative bacteria isolates, *E. coli* and *K. pneumonia* were predominant with percentages 83 (41.5%) and 107 (53.5%), respectively. However, proteus was 10

(5%). Most of the isolated Enterobacteriaceae were from urine with a frequency of 55 (54.46%) and 43(42.57%) for *E. coli* and *K. pneumonia*, respectively. There was a significant association between isolated bacteria and sources of the sample, with P value< 0.05.

Table 3: Sample wise occurrence of isolates.

Isolate	*Pus*	*Urine*	*Sputum*	*Blood culture*	*Body fluids*	*Total*
E. coli	23(31.1)	55(54.5)	0	03(18.8)	02(66.7)	83(41.5)
Proteus	06(8.1)	03(2.9)	0	01(6.3)	0	10(5)
Klebsiella pneumonia	45(60.8)	43(42.6)	06(100)	12(75)	01(33.3)	107(53.5)
Total	74(37)	101(50.5)	06(3)	16(8)	03(1.5)	200(100)

Chi-square value = 20.75, p value = 0.007

The table 4 shows that out of 200 Gram-negative bacteria isolates, 89(44.5%) were ESBL positive and 111(50.5%) were ESBL negative. There was a significant association between isolated bacteria and ESBL, with P value < 0.05.

Table 4: Organism wise distribution of ESBL.

Isolate	*ESBL +ve*	*ESBL –ve*	*Total*
E.coli	33(39.8)	50(60.3)	83(41.5)
Proteus	01(10)	09(90)	10(5)
K. pneumonia	55(51.4)	52(48.6)	107(53.5)
Total	89(44.5)	111(50.5)	200(100)

Chi-square value = 7.64, p value = 0.02

As seen from the table 5 maximum no. of ESBL +ve isolates were obtained from surgery ward followed by orthopedic ward. Among the different clinical samples ESBL producers were found most commonly in pus 58.66% (44/74) & urine 36.63% (37/101). Maximum number of ESBL +ve *E.coli* isolates were obtained from medicine ward and maximum no of *E.coli* isolated from Urine sample (26/33). ESBL +ve *Proteus* isolated only from CCU and it is from blood sample. Maximum number of ESBL +ve *Klebsiella pneumoniae* isolates were obtained from surgery ward and from pus sample (38/55).

Table 5: Ward wise and clinical specimen wise distribution of ESBL +ve based on clinical samples, *E.coli*, *Proteus, Klebsiella pneumoniae* & its prevalence in each ward.

Isolates	*Sample*	*Surgery*	*Medicine*	*NICU*	*BMW*	*CCU*	*Orthopedics*	*ESBL + ve (%)*
Clinical sample	Urine	18	4	1	6	3	5	37(36.63)
	Pus	32	0	0	0	0	12	44(58.66)
	Blood	1	2	0	1	1	0	5(31.25)
	Sputum	0	2	0	0	0	0	2(2.04)
	Body fluid	1	0	0	0	0	0	11.02)
E.coli	Urine	3	15	0	5	1	2	26(78.79)
	Pus	6	0	0	0	0	0	6(18.19)
	Blood	0	0	0	0	1	0	1(3.03)
	Sputum	0	0	0	0	0	0	0
	Body fluid	0	0	0	0	0	0	0
Proteus	Urine	0	0	0	0	0	0	0
	Pus	0	0	0	0	0	0	0
	Blood	0	0	0	0	1	0	1(100)
	Sputum	0	0	0	0	0	0	0
	Body fluid	0	0	0	0	0	0	0

Klebsiella pneumoniae	Urine	10	3	0	1	1	1	16(29.09)
	Pus	30	0	0	0	0	8	38(69.09)
	Blood	0	0	0	0	1	0	1(1.82)
	Sputum	0	0	0	0	0	0	0
	Body fluid	0	0	0	0	0	0	0

The table 6 shows that ESBL strains are 100% resistant to 3rd generation cephalosporin, 75(84.26%) to gentamycin, 71(79.77%) resistant to ciprofloxacin, 63(70.78%) resistant to Amikacin, 10(11.23%) resistant to ampicillin/ sulbactam, 40(45%) resistant to and all strains are sensitive to imipenem, meropenem, cefoperazone/ sulbactam and ceftriaxone.

Table 6: Antibiotic susceptibility pattern in ESBL producer.

Drug name	*n (%) of resistance*	*n (%) of sensitive*
Ceftazidime (CAZ)	89(100)	0
Cefuroxime (CFR)	89(100)	0
Cefoperazone (CPZ)	89(100)	0
Cefotaxime (CTX)	89(100)	0
Ceftriaxone (CTR)	89(100)	0
Amikacin (AK)	63(70.78)	26(29.21)
Gentamycin (GEN)	75 (84.26)	14(15.73)
Ampicillin + sulbactam (AS)	10(11.23)	79(88.76)
Tetracycline (TE)	40(45)	49(55.05)
Ciprofloxacin (CIP)	71(79.77)	18(20.22)
Imipenem (IPM)	00	89(100)
Meropenem	00	89(100)

Discussion

ESBL production by Gram-negative bacteria has become a major problem in clinical practice in last few years due to extensive use of the beta-lactam antibiotic. The chromosomally mediated beta-lactamases are inducible or constitutive non-transferable. The second type of beta-lactamases is the plasmid mediated ESBLs, which are constitutive expressed and transferable. Cotransfer of resistance against aminoglycoside, trimethoprim, sulfonamides, tetracycline, chloramphenicol and quinolones is also common on ESBL plasmids [14].

There is ongoing debate about the optimal treatment of patients infected with ESBL producing bacteria and the actual *in vivo* activity of various third and fourth generation cephalosporin antibiotics against these bacteria. A strict recommendation has been published rejecting the use of third and fourth generation cephalosporin against ESBL producing bacteria resulting vastly increased use of carbapenems or non-beta-lactam agents [15].

Cefepime use for the systemic infections caused by ESBL producing bacteria may fail due to selection of ESBL producing bacteria during treatment and several studies have documented clinical failures. Therefore, cefepime act against ESBL producing is not recommended unless given in high dose and combined with amino glycoside or quinolone [19]. Prospective studies of efficacy of third or fourth generation cephalosporin's for such infection will probably never be conducted due to the aforementioned recommendations and would probably even be considered unethical today [15].

The present study included only those organisms which showed decreased susceptibility or were resistant to any of the third generation cephalosporin. In our study main sources of ESBL producing strains were pus, urine, & blood. ESBL is mainly produced by *Klebsiella pneumoniae* & *E.coli* in this study. The percentage of ESBL producing *Klebsiella pneumoniae* 51.39%, *E.coli* 39.75% & *Proteus mirabilis* 10%. In the present study 89(44.5%) of the isolates were ESBL positive i.e. found to be ESBL's producer out of 200 Gram negative isolates from different samples. However, a similar study which was observed in 2009 and in 2007 in the institute, BSMMU, by Biswas et al. and Rahman revealed ESBLs in 66.36% and 30.90% strains of the Gram negative isolates respectively [16, 17]. However, in a 2012 study done by Yasmin, Bangladesh detected 71.4% ESBLs producers from 300 Gram negative isolates [18].

In present study ESBL producers were sensitive to imipenem, meropenem, cefoperazone/ sulbactam and ceftriaxone. Which is similar to the study done by Perez et al. that carbapenems (e.g., imipenem, meropenem, and ertapenem) have the most consistent activity against ESBL-producing Enterobacteriaceae [19]. Large scale surveillance studies demonstrate that >98% ESBL-producing *E. coli*, *Klebsiella* spp, and *P. mirabilis* isolates are susceptible to carbapenems [20-22].

Based on retrospective and prospective analyses, carbapenems should be considered as the preferred treatment for infections due to ESBL-producing Enterobacteriaceae [23]. There is no evidence that combination therapy with a carbapenems and antibiotics of other classes is superior to the use of carbapenems alone [24].

The choice between imipenem and meropenem is difficult. Intrathecal polymyxin B should also be considered along with removal of neurosurgical hardware in cases of CSF shunt infections [25]. In Menon et al study, all the isolates were sensitive to imipenem and piperacillin-Tazobactam [26]. However in present study also all the isolates were sensitive to imipenem as well as meropenem.

Various studies report *in vivo*, ertapenem susceptibility of clinical isolates. Few authors tested 181 ESBL producing clinical Enterobacteriaceae isolates (all *Klebsiella* spp) taken from ICU patients and found ertapenem to inhibit 90% of isolate which correlates with our study [27, 28].

Kumar et al. found 72% isolates were resistant to gentamycin and 93% were resistant to ciprofloxacin. They have found 95% of the isolates were resistant to cefotaxime, whereas in the present study, there was 100% resistance to cefotaxime, 83% resistance to gentamycin and 80.22% resistance to ciprofloxacin because plasmid mediated ESBLs, which are constitutively expressed and transferable [29].

In present study, it was found that 83% of ESBL producers were resistance to gentamycin, which is proximately same as the study done by Babypadmini et al., and Sharma et al [30, 31].

The situation may change from place to place, thus institutional antibiograms or local patterns of susceptibility should be used to decide the choice of drugs.

Conclusion

The present study included only those organisms which showed decreased susceptibility or were resistant to any of the third generation cephalosporin. In our study main sources of ESBL producing strains were pus, urine, & blood. ESBL is mainly produced by *Klebsiella* & *E.coli* in this study. The percentage of ESBL producing *Klebsiella* 51.39%, *E.coli* 39.75% & *Proteus mirabilis* 10%. ESBL strains showed high degree of resistance to 1st, 2nd & 3rd generation cephalosporin like cefuroxime, cefotaxime, ceftazidime etc. ESBLs have become an extensive serious issue. Several aspects of ESBLs are worrying. These enzymes are becoming progressively expressed by various strains of pathogenic bacteria with a potential for dissemination.

Recommendation

For treatment of patients infected with ESBL producing strains, clinicians are suggested to use antibiotics like β-lactam/ β-lactamase inhibitor combination like cefoperazone/ sulbactam, ceftazidime/ clavulanic acid, piperacillin/ tazobactam & carbapenems like meropenam and Imipenem. Awareness amongst clinician is a must and data needs to be projected so that choice of appropriate antibiotic is done for patient treatment.

Limitations

Main limitation of this perspective cross-sectional study was that it was conducted in one district of Gujarat, which is not sufficient to reflect the epidemiology of different geographical areas or different states.

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Conflicts of interest

Author declares no conflicts of interest.

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